Distribution and Ecology of *Pythium* Species in Japan

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摘　　要

Watanabe, T. (1985) 日本猝倒病菌 (***Pythium***) 之分布與生態 (日本農林水產省林業試驗本場)

本文所討論之日本產*Pythium* spp. 之分佈及生態是1968～1984年間由日本各地採取80種植株根部及 263 種土壤樣品分離所得之試驗結果。植株根部組織經洗淨後置於水瓊脂平板上，由長出之*Pythium* spp. 之菌絲尖端移植培養後供試。以修正過的Waksman之直接接種法，或是用胡瓜種子等供做土壤分離 *Pythium* spp. 用。分離時至少在二種不同溫度下進行，一是在 10°C 以下，另一是置於 20°C 以上，放置 1 至 7 天。試驗結果顯示與植物根部有密切關聯之優勢種類順序爲 *P. sylvaticum*, *P. spinosum*, *P. aphanidermatum* 和 *P. ultimum*，上述每一種類皆由 10 個以上之樣品分離而得。*Pythium* spp. 普遍存於日本國內土壤。在 263 個樣品中有 247 土樣 (佔 93.9%) 有 *Pythium* spp. 之存在，其中四種最常見者與根部密切關聯之優勢種相同。第五群之 *Pythium* spp. 僅形成類似菌絲之孢子囊及游走孢子，而沒有性器官，暫以 H-Zs (hyphae-zoospores) 名之，此群之分離出現頻率順序爲 *P. irregulare*, *P. vexans*, *P. splendens* 及 *P. torulosum*。優勢種因不同地區而稍有差異，但一般年平均溫高的地方 *P. aphanidermatum* 出現較多。所有供試之每一土樣可分離到 2 至 3.4 種 *Pythium* spp., 平均爲 2.6 種。以修正過的 Waksman 直接接種法分離 *Pythium* spp. 之量發現，由 Tohoku 及琉球羣島採取之 48 種土樣，其中之 12、17 及 19 種土樣每克土壤中可分得 201 以上，51～100 及少於 50 之 *Pythium* spp. 繁殖體。

ABSTRACT

Distribution and ecology of *Pythium* species in Japan were discussed on the basis of data obtained by assaying 80 plant root samples and 263 soil samples collected in various districts in Japan during 1968-1984. *Pythium* cultures were obtained from plant roots by placing washed tissue pieces on plain water agar plates and single hyphal tips from the fungal growth were

cut and transferred for further cultural works. Either a modification of Waksman's direct inoculation method or trapping with cucumber seed and other substrates was used to isolate *Pythium* spp. from soil. All isolation works were carried out at least at two temperature levels, with one below 10 C and one above 20 C. One to 7 days of incubation was allowed. The dominant species associated with plant roots were *P. sylvaticum*, *P. spinosum*, *P. aphanidermatum*, and *P. ultimum* in the descending order. These species were each isolated from more than 10 samples. *Pythium* species were ubiquitous in Japanese soils, being detected in 247 (93.9%) out of 263 samples. The four most dominant species were same as those from plant roots. A fifth group of *Pythium* which formed only hypha-like sporangia and zoospores, but had no sexual organs, was tentatively named H-Zs, an abbreviation for hyphae-zoospores. This was followed by *P. irregulare*, *P. vexans*, *P. splendens*, and *P. torulosum* in frequency of isolation. The dominant species was somewhat different with districts. *Pythium aphanidermatum* was more frequently detected in districts with a high yearly average of air temperature. Based on total soil samples the average yield was 2.6 species per sample while the average yield ranged from 2 to 3.4 species per soil sample, depending on districts. In assaying population of *Pythium* spp. by a modification of Waksman's direct inoculation method, 12, 17, and 19 out of 48 soil samples from Tohoku and the Ryukyu Islands yielded a total number of propagules exceeding 201; in the range of 51-200 and fewer than 50 per g. of soil, respectively.

INTRODUCTION

Although more than 200 literature have been published on *Pythium* diseases of various plants in Japan, only a few reports dealt with the distribution and ecology of *Pythium* species. In the course of an etiological study of soilborne diseases of higher plants in Japan, data on *Pythium* spp. as well as on diseases they incited have been accumulated. I shall discuss the subject on the basis of these data.

Identification of isolates.

Of the nearly 4,000 isolates studied some were difficult to identify, because they did not form sexual organs and showed no other distinctive morphological characteristics, except spherical or filamentous sporangia. Zoospores were never produced by isolates with spherical sporangia. A second group of fungi was provisionally named H-Zs, an abbreviation for hyphae-zoospores, because they formed zoospores from hypha-like sporangia. The zoospore-nonproducer isolates were tentatively identified as *P. sylvaticum* Campbell et Hendrix[2]. They included those that were definitely *P. sylvaticum*. The H-Zs isolates could well be *P. flevoense* Van Der Plaats-Niterink[14], a heterothallic species with hypha-like sporangia.

Table 1 lists the 31 species of *Pythium*, including H-Zs, recorded from the this study, together with the representative isolates.

Table 1. *Pythium* species and the representative isolates studied

Oogonia (echinulate)

1. *P. acanthicum*: 80-228 (Cabbage field, Kagoshima); 81-256 (Eggplant field, Fukushima)
2. *P. echinulatum*: 822 (Strawberry, Saitama, ATCC 36437): 81-219 (Radish field, Kanagawa)
3. *P. irregulare*; 78-2452 (Peanut field, Aomori) 81-30 (Cabbage field, Kanagawa)
4. *P. periplocum*(?): 78-2243 (Watermelon field, Aomori)
5. *P. spinosum*: 160 (Strawberry, Shizuoka, ATCC 36438); 79-468 (Eggplant field, Kagoshima)

Oogonia (smooth)

A. Hypha-like sporangia

6. *P. angustatum*: 257 (Strawberry, Shizuoka, ATCC 36485)
7. *P. apleroticum*: 841 (Strawberry, Saitama, ATCC 36441)
8. *P. dissotocum*: 81-708 (Lettuce field, Chiba)
9. *P. monospermum*: 80-571 (Pea field, Kagoshima)
10. *P. sulcatum*: 83-160 (Carrot, Chiba, Nagai's isolate)

B. Lobulate sporangia

11. *P. aphanidermatum*: 54 (Strawberry field, Shizuoka, ATCC 36431): 79-71 (Cucumber, Okinawa)
12. *P. deliense*: 79-76 (Pumpkin, Okinawa); 79-226 (Adzuki bean field, Kagoshima)
13. *P. graminicolum*: 80-214 (Soybean field, Miyazaki); 80-302 (Corn field, Kumamoto)
14. *P. inflatum*: 51 (Strawberry field, Shizuoka, ATCC 36436)
15. *P. myriotylum*: 864-1 (Strawberry, Saitama ATCC 36440); 83-193 (Chinese arrowhead, Saitama)
16. *P. torulosum*: 256 (Strawberry, Shizuoka, ATCC 36484); 81-327 (Grassland, Hokkaido)
17. *P. zingiberum*: 82-569 (Ginger, Wakayama); 82-724 (Ginger field, Wakayama)

C. Spherical sporangia

18. *P. nelumbium*: 84-209 (Bamboo field, Kohchi)
19. *P. oedochilum*: 165 (Strawberry, Shizuoka, ATCC 36433); 82-634 (Spinach, Nara)
20. *P. paroecandrum*: 827 (Srawberry, Saitama, ATCC 36432); 83-400 (Forest soil, Shizuoka)
21. *P. pulchrum*: 79-33 (Watermelon field, Aomori); 81-386 (Corn field, Hokkaido)
22. *P. rostratum*: 81-424 (Potato field, Hokkaido): 82-143 (Melon field, Chiba)
23. *P. splendens*: 192 (Strawberry, Shizuoka, ATCC 36444); 82-17 (Melon, Chiba)
24. *P. sylvaticum*: 866 (Strawbesry, Saitama, ATCC 36442); 80-164 (Watermelon field, Fukuoka)
25. *P. ultimum*: 901 (Strawberry, Saitama, ATCC 36443); 80-225 (Sweet potato field, Kagoshima)
26. *P. vexans*: 79-203 (Chinese cabbage field, Kagoshima); 80-274 (Cucumber field, Fukuoka)

No. sexual organ formed

27. *P. afertile*: 663 (Strawberry, Shizuoka, ATCC 36439)
28. *P. carolinianum*: 5 (Strawberry field, Shizuoka, ATCC 36434); 79-170 (Cabbage field Okinawa)
29. *P. elongatum*: 79-72 (Tomato, Obinawa)
30. *P. intermedium*: 483-2 (Strawberry field, Shimane ATCC 36445); 81-395 (Forest soil, Hokkaido)
31. H-Zs: 81-402 (Corn field, Hokkaido); 82-692 (Radish field, Osaka)

Pythium species associated with higher plants.

For isolation of *Pythium* from plant roots, tissue pieces (less than 5 mm long) dissected from random root samples were washed in running tap water for 0.5-1 h, blotted, and placed on water agar plates without previous surface sterilization. Hyphal tips were selected at random from the fungal growth from each tissue piece after 1-7 day incubation at two temperature levels, one below 10 C and one above 20 C, and were transferred to PDA plates for further cultural works.

A total of 682 *Pythium* cultures were obtained by assaying 80 root samples from 35 higher plants collected in various districts in Japan during 1968-1984. They were classified into at least 24 species, and the most dominant species were *P. sylvaticum*. *P. spinosum*, *P. aphanidermatum* and *P. ultimum* in the descending order (Table 2). They were each

Table 2. Detection and isolation of *Pythium* spp. from plant roots in Japan

Location (District)	Host	Sample no.	Isolate no.	No. of samples with Pythium[1)]				
				Pa	Pspi	Psyl	Pu	Others
Hokkaido	Azzuki bean, Bean	2	6	0	0	2	0	2
Tohoku	Cucumber and 7 others	8	43	4	2	4	2	4
Kanto	Strawberries and 10 others	24	280	4	5	5	1	15
Chubu	Strawberries and 6 others	12	109	0	6	5	2	8
Kinki	Strawberries and 6 others	11	111	1	3	3	4	7
Chugoku	Ginger and 4 others	4	28	0	1	1	1	4
Shikoku	Tomato and 3 others	3	4	0	1	1	0	1
Kyushu	Cucumber and 11 others	16	101	8	2	2	2	7
Total		80	682	17	20	23	12	48

1) Pa=*P. aphanidermatum*, Pspi=*P. spinosum*, Psyl=*P. sylvaticum*
Pu=*P. ultimum*, Others=P. deliense and miscellaneous species.

detected and isolated from more than 10 samples. These species had a wide host range[8], and several *Pythium* spp. could be detected from a single plant species. For example, 14 *Pythium* species were found associated with strawberry roots that were examined for the purpose of determining the etiology of a new stunt disease[24]. Data were summarized in Table 3. In the second example,

Table 3. *Pythium* species and number of isolates from strawberry roots and the rhizosphere soils in Japan Watanabe et al.(1977)

	No. of isolates			No. of isolates	
	Roots	Soils		Roots	Soils
P. afertile	2	—	*P. myriotylum*	2	—
P. angustatum	4	1	*P. oedochilum*	40	—
P. aphanidermatum	—	16	*P. paroecandrum*	3	—
P. apleroticum	1	—	*P. spinosum*	42	14
P. artotrogus (?)	5	6	*P. splendens*	3	9
P. carolinianum	—	49	*P. sylvaticum*	138	12
P. echinulatum	1	2	*P. torulosum*	2	—
P. inflatum (?)	1	—	*P. ultimum*	46	1
P. intermedium	—	1	*Pythium* sp.	1	—
TOTAL				291	111

diseased cucumber plants collected at Yamagata in 1978 were found to harbor *P. aphanidermatum* and four other species, but several samples collected in other areas yielded only *P. aphanidermatum*[20]. In another example, *P. splendens* was most frequently isolated from roots of wilted melon in vinyl houses in Chiba prefecture[25], but *P. irregulare* and *P. spinosum* were very rarely isolated from these materials. Samples yielding the latter fungi were from different locations at different sampling dates.

Ubiquitous distribution in soils.

Although nearly 40 species of *Pythium* were isolated and identified from higher plants, algae, and water before 1970, there has been little work on their isolation directly from soil. Therefore, the isolation of *Pythium* from the strawberry rhizosphere soils may be significant (Table 3).

Isolation from soils was conducted either by a modification of Waksman's direct inoculation method or by trapping. In the former case, less than 2 mg of soil samples was placed on plain water agar plates, and after incubation for 24-48 hr, pure isolates were obtained by single hyphal tippings from suspected *Pythium* colonies.

In trapping, soil samples weighing 10 to 50 g were mixed with trapping substrates. Sufficient water was added to keep petri dishes in a flooded condition. Potato discs, 4 mm in diam, were used as trapping substrates before 1978 and cucumber seeds were used after 1978. Following an incubation period of 1-7 days at a temperture below 10 C or above 20 C, the substrates were removed, washed under running tap water over 30 min., blotted to remove surface water, and placed on plain water agar plates. Further procedures were same as in the Waksman's direct inoculation method.

A total of 263 soil samples were collected from farmlands rice paddy

Table 4. Detection and isolation of *Pythium* spp. from soils in various districts of Japan

Location (District)	1968—1977				1978—1984			
	No. of samples		Rate of detection %	No. of isolates obtained	No. of samples		Rate of detection %	No. of isolates obtained
	Tested	Detected			Tested	Detected		
Hokkaido	7	7	100	65	21	20	95.2	250
Tohoku	—	—	—	—	28	26	92.9	221
Kanto	10	10	100	833	21	20	95.2	276
Chubu	57	54	94.7	386	12	8	66.7	111
Kinki	1	1	100	2	28	26	92.9	238
Chugoku	6	3	50	6	—	—	—	—
Shikoku	—	—	—	—	26	26	100	237
Kyushu	—	—	—	—	36	46	100	619
Total	81	75	92.6	1292	182	172	94.5	1952

fields, grasslands or forests in various districts in Japan in more than 40 occasions since 1968. Some of the 81 samples collected before 1978 were assayed only by the direct inoculation method, while trappind with cucumber seeds was used exclusively since 1978. *Pythium* species were detected in nearly 94% of the 263 soil samples assayed (Table 4). Sixteen samples yielding no *Pythium* propagules might be free from the fungi or otherwise the fungal populations must have been too low to be detected. However, it is certain that *Pythium* species could be detected in most soils in any district of Japan by the trapping method using cucumber seed or other substrates.

Dominant species in soils and number of species per sample.

At least 25 species of *Pythium* were detected or isolated from soil from all over Japan. The number of detected species varied with districts, ranging from 10 species in Hokkaido to 17 in Kyushu. *Pythium sylvaticum* was the most widely distributed species, being detected in 125 out of 182 soil samples collected in 1978 to 1984. Other dominant species were *P. spinosum*, *P. aphanidermatum* and *P. ultimum* in the descending order. If the sample number of fungus detection to the total number of samples assayed is defined as detection rate, that of *P. sylvaticum* was 68.7% and those for the latter 3 species ranged from 24 to 27%. The dominant species in different soils are illustrated, together with the detection rates in Fig. 1.

The number of species present in a soil sample differed with samples. Of 182 samples assayed an average of 2.6 species per sample was obtained (Fig. 2). However, as many as 6 species were detected per sample in 5 samples. By district, the average yield was two to 3.4 species per sample.

Pythium sylvaticum was usually the most frequently detected from each district. Other dominant species were encountered less consistently, For example, the dominat species were *P. sylvaticum*, *P. aphanidermatum*, and *P.*

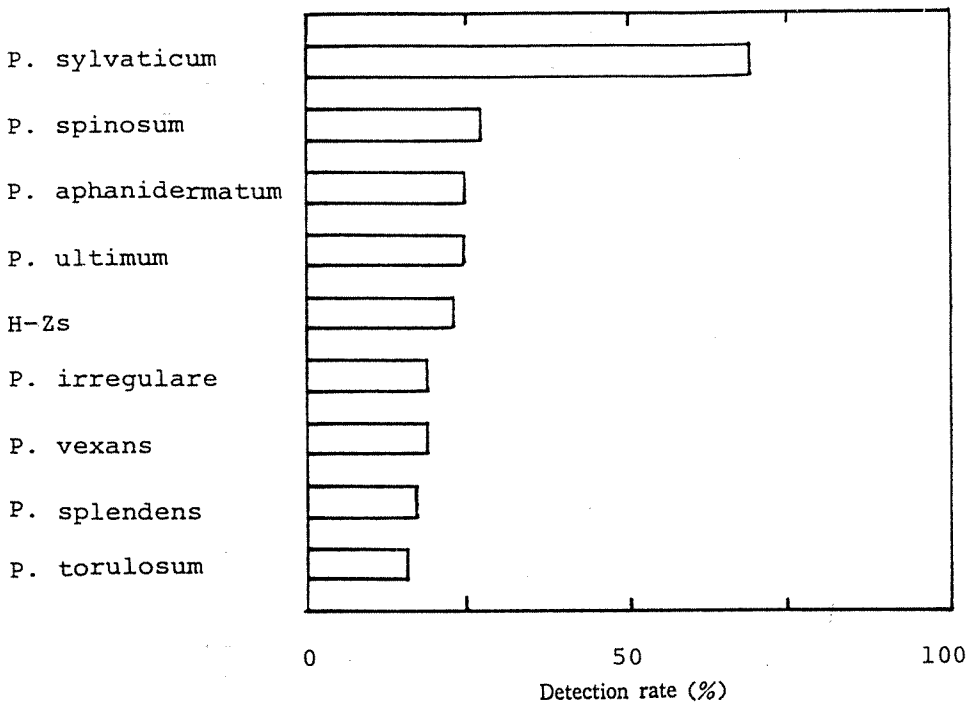

Fig. 1. Dominant *Pythium* species found in Japanese soils and their detection rates. Samples of 1978-1984.

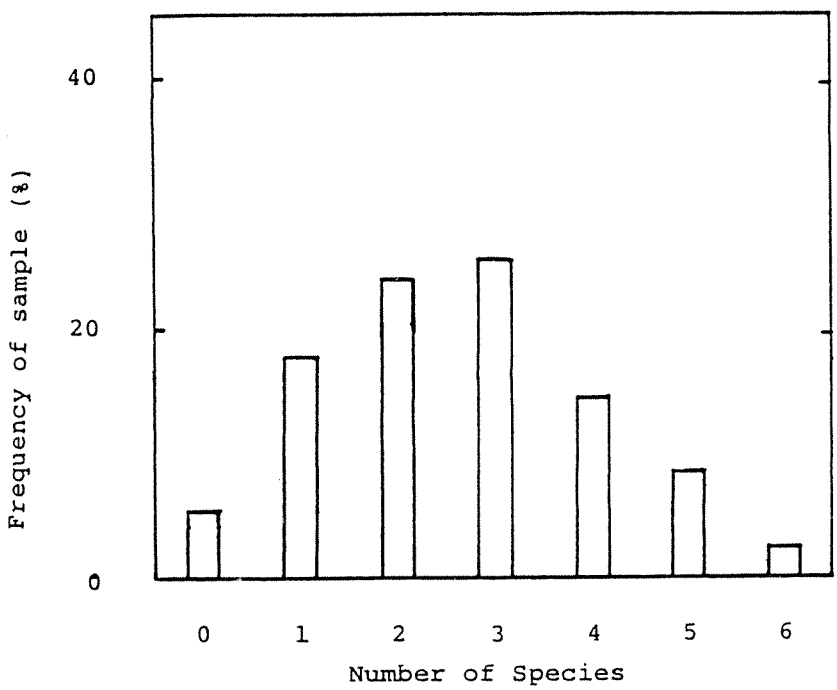

Fig. 2. Frequency (%) of soil samples with indicated numbers of *Pythium* species in Japan

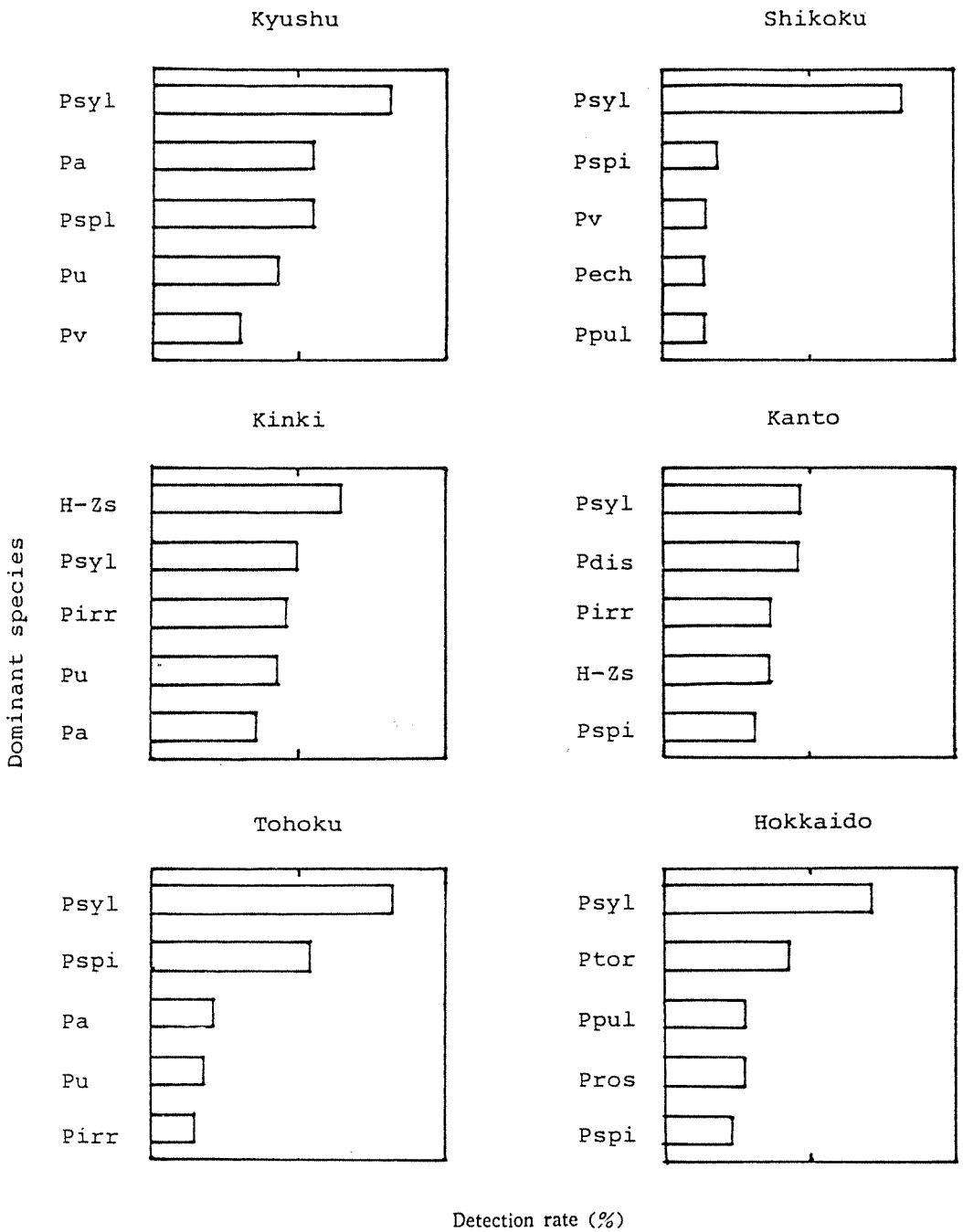

Fig. 3. Dominant *Pythium* species found in soils from six districts of Japan and their detection rates. Samples of 1978-1984. Pa=*P. aphanidermatum*, Pdis =*P. dissotocum*, Pech=*P. echinulatum*, Pirr=*P. irregulare*, Ppul=*P. pulchrum*, Pros=*P. rostratum*, Pspi=*P. spinosum*, Pspl=*P. splendens*, Psyl =*P. sylvaticum*, Ptor=*P. torulosum*, Pu=*P. ultimum*, Pv=*P. vexans*, H-ZS=a group of fungi with hypha-like sporangia, but without sexual organs.

Table 5. Incidence of soil-borne diseases of cucumber seedlings[1] in relation to populations of *Pythium* spp. in soils collected in six prefectures in southern and northern Japan

Based on Watanabe (1981, 1983)

Location (Prefecture)	Population (Propagules/g soil)	Sample no.	Disease incidence Average (%)
Okinawa	216.6[a]	11	94.5[ac]
Kagoshima (Amami-Ohshima)	62.1[a]	10	56.8[b]
Akita	215.9[a]	8	70.8[bc]
Aomori	123.8[a]	10	57.4[b]
Iwate	72.7[a]	5	69.4[bc]
Yamagata	20·2[a]	4	65 [bc]

1) No. of collapsed seedlings (no. of ungerminated seeds included)/no. seeds sown x 100. Numbers followed by the same letter do not differ significantly (P=0.01).

Table 6. Populations of *Pythium* spp. in soils

Location	Habitat and sample no.	Population	Literature
ENGLAND			
Kennington	Forest nursery (4)	55-687/g	Warcup (1952)[16]
Australia			
Southern Aust.	Forest nursery (18)	24-226/cm^3	Vaartaja & Bumbieris (1964)[13]
Adelaide	Pea, uncultivated land (4)	16-343/g	Kerr (1963)[6]
CANADA			
Ontario	Forest nursery (34)	0-92.5/cm^3	Vaartaja (1968)[12]
U. S. A.			
Southeastern US	Forest nursery (70)	0-179/g	Hendrix & Campbell (1968)[3]
California	Arable land (?)	64-3814/g	Stanghellini & Hancock (1970)[11]
Arizona	Arable land (4)	10-250/g	Burr & Stanghellini (1975)[1]
Maryland	Peach orchard (?)	64-378/g	Mircetich & Fogle (1969)[9]
Maryland	Arable land (5)	0-558/g	Lumsden et al. (1975)[7]
JAPAN			
Ryukyu Islands	Arable land (21)	0-1040/g	Watanabe (1981)[20]
Tohoku	Arable & uncultivated land (27)	0-441/g	

splendens in Kyushu, H-Zs and *P. sylvaticum* in Kinki, and *P. sylvaticum* and *P. spinosum* in Tohoku. In Shikoku and Hokkaido districts, *P. sylvaticum* was again dominant (Fig. 3).

Distribution in relation to temperature responses.

Pythium aphanidermatum, *P. deliense* and *P. myriotylum* need high temperatures, 34-37 C, for the optimum vegetative growth[8,9]. They have been thought to occur only in warm areas. However, in this study, *P. aphanidermatum* was found present in 52.4% of the samples from the Ryukyu Islands, where the average air temperature is above 20 C, and in 48% of the test samples from Kyushu where the average air temperature ranges from 14 to 17 C. In the Tohoku district where the yearly average of air temperature is 10 C, the fungus was detected in 4 out of 27 samples assayed. No fungus was detected in 21 samples from Hokkaido where average air temperature goes below 8 C. Therefore, the detection rates of *P. aphanidermatum* increased with the increase in average annual air temperature for districts[22] (Fig. 4).

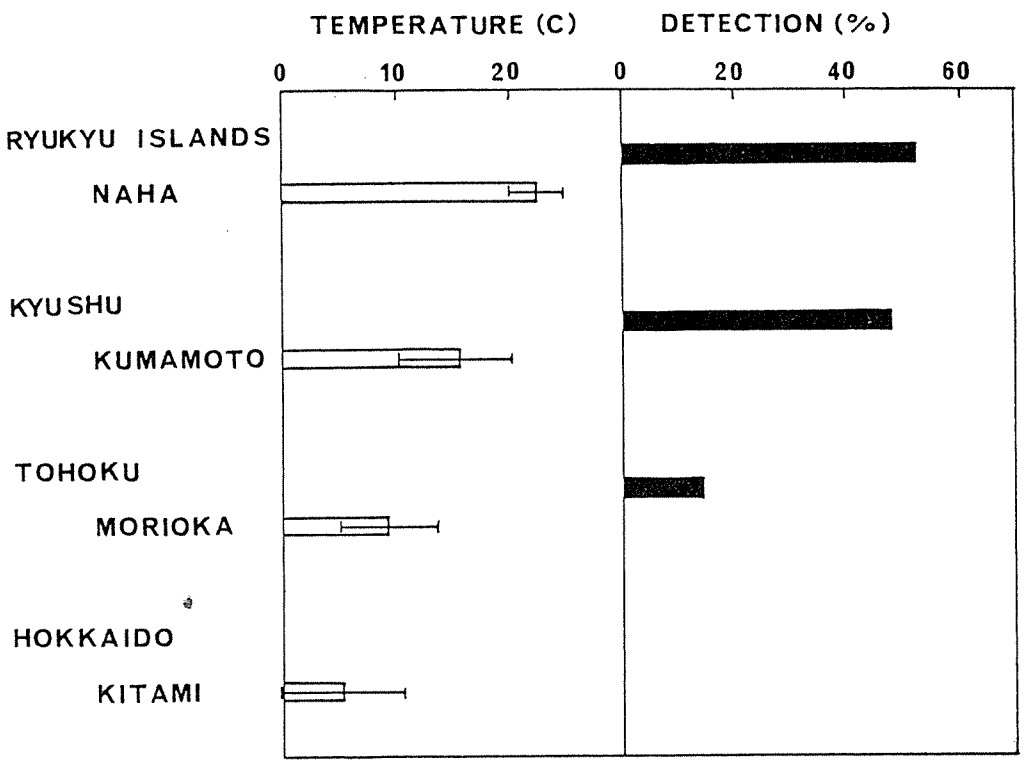

Fig. 4. Detection rates (%) of *Pythium aphanidermatum* in soil samples from the Ryukyu Islands and Kyushu in southern Japan and from Tohoku and Hokkaido districts in northern Japan in relation to the average annual air temperature in each area. Each thick bar represents the average annual air temperature with the maximum and minimum temperatures indicated by the solid line. Watanabe (1983)

The distribution of *P. deliense* appears to be limited to the Ryukyu Islands, and this fungus was isolated from pumpkin, tomato and bean roots, and also from five soil samples[21]. *Pythium. myriotylum* was isolated from strawberry[18,24] dasheen [10] and Chinese arrowhead[26] in Kanto district, but noteworthily it was also found on beans and in soil at Kitami of Hokkaido district[5].

In contrast to other 15 species from strawberry roots and the rhizosphere soils which had an optimum temperature at or above 28 C, *P. intermedium* grew best vegetatively at 25 C[19]. This fungus was detected in soils from various districts including Hokkaido and Mt, Fuji, especially by trapping made at temperature below 10 C.

Fungal populations and disease incidence in cucumber seedlings.

Only a few reports are available on the populations of *Pythium* in Japanese soils.

In this study, populations of *Pythium* spp. were determined in soil samples from Tohoku and the Ryukyu Islands, using a modification of Waksman's method[20]. Out of 27 samples from Tohoku assayed, nine samples each yielded more than 201 propagules per g of soil, 8 samples each yielded 51-200 propagules and 10 samples each yielded fewer than 50, whereas in soil samples from the Ryukyus, these propagule levels were found respectively in 3, 9 and 9 samples out of 21 tested. Dominant species composing these populations in Tohoku soils were *P. sylvaticum* and *P. spinosum*, and in the Ryukyu soils, it was *P. aphanidermatum*.

The highest propagule number was recorded in Okinawa soils, with an average of 1,039 per g of soil. All of the propagules belonged to *P. deliense*. Prefecturewise, Okinawa soils had an average of 216.6 per g of soil. The detail assay results are shown in Table 5, together with disease incidence data of cucumber seedlings. For comparison, similar data from the United States and other countries are given in Table 6.

We also determined disease incidence of cucumber seedlings as affected by *Pythium* populations in the soil samples in a previous work, Five seeds were sown in each of 50 g soil per dish with 30 ml of water, and incubated at 22 C. The collapsed plants were examined after an incubation period of 12 days.

The disease incidence differed statistically between Okinawa and Kagaoshima, and also between Okinawa and Aomori, but there was no significant difference when other prefectures were compared. Although pathogenicity test was not made in this study for each *Pythium* isolate. *P. aphanidermatum*, a pathogen of cucumber seedlings abundantly present in Okinawa soils, is believed to be closely related to the collapse of cucumber seedlings in Okinawa.

DISCUSSION

Pythium species were often found in roots of various plants, together with *Fusarium* and *Rhizoctonia* species in Japan [17]. Therefore, one tends to relate the *Pythium* isolates recovered to the soil-borne diseases observed, but this approach must be taken with caution, because the fungi are ubiquitous in soils and some of them may not be pathogenic.

The dominant species associated with roots were quite similar to those present in soils in Japan. Likewise, these fungi were also recorded as dominant in the soils of the continental United States[4].

Recently, a simple and reliable method was developed for detection and quantitative estimations of *P. aphanidermatum* in soil[23]. Further improvement is needed for better under standing of the distribution and ecology of *Pythium* species in soil and for taxonomic clarification of those ambiguous species in the group.

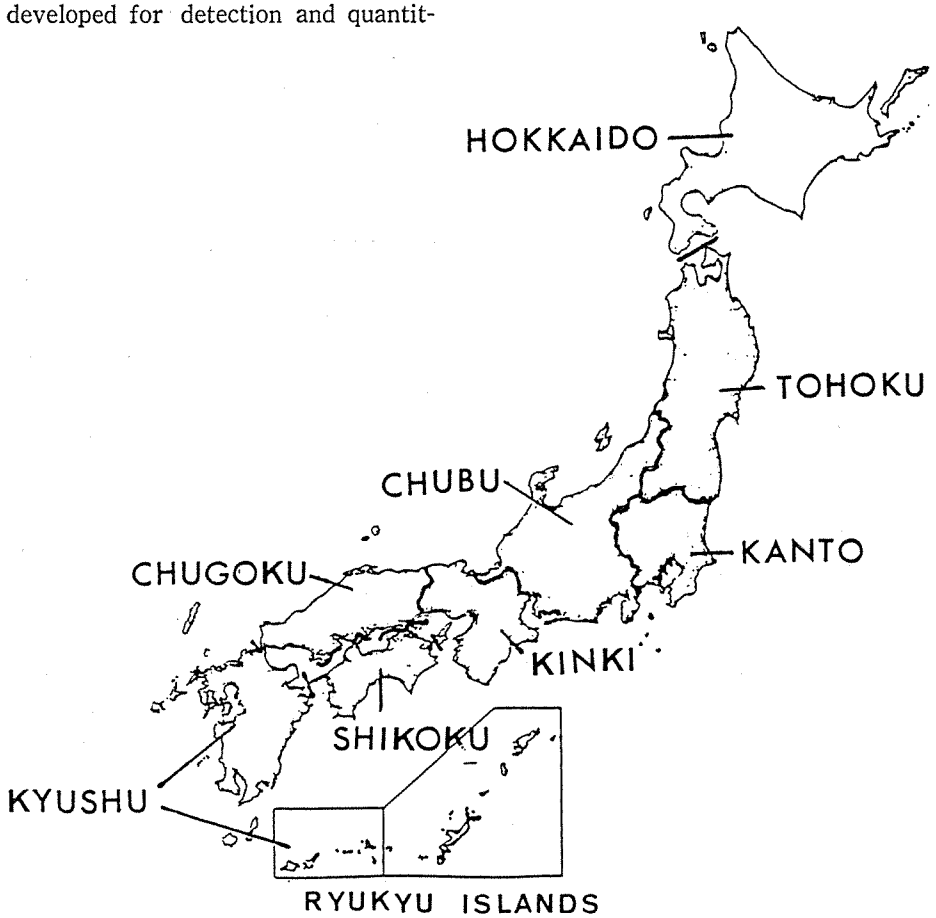

Fig. 5. Map of Japan

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